Amendments to the Specification

Please replace paragraph 0036 on pages 12-13 with the following:

FIGs. 3A and 3B depict schemes showing preparation of a TMR-labeled protein by coupling an organic thioester labeled with a fluorescent dye such as tetramethylrhodamine (Segment A) to a protein with N-terminal cysteine (Segment B). FIG. 3A depicts a scheme for forming a labeled protein by acylating triethylenetetramine (TREN, available from Aldrich, Milwaukee, WI, Catalogue No. 90462) with 3.5 equiv. of an activated ester of carboxytetrarhodamine (TMR), available from Molecular Probes, OR (Catalogue No. e-6123), to form $(TMR)_3$-TREN **5**. Acylation of N^{α}-*Fmoc*-Lysine with 2-iminobiotin-N-hydroxysuccinimide ester (Biotin-NS ester) yields N_{ε}-*Fmoc*-N^{α} -biotin-Lysine **6**. Deblocking of the ~~α-amino~~ ε-amino group of **6** followed by acylation with bromoacetyl chloride forms N_{ε}-bromoacetamido- N^{α}-biotinyl-Lysine **8**. The carbodiimide coupling of **8** with α-toluenethiol results in **9**. The alkylation of **5** with the thioester **9** in the presence of sodium iodide generates the quaternary ammonium salt **10** (Segment A) that upon coupling with Segment B under the same conditions described above affords **11** (chromophore to protein ratio = 3). FIG. 3B depicts a scheme for forming a TMR-labeled protein by first preparing a thiol benzyl ester (**13**). Deprotection of the amino group of **13** in the presence of trifluoroacetic acid, **14**, followed by coupling to N-hydroxy succinimidyl ester of TMR generates the benzyl thioester derivative of N-TMR-8-heptanoic acid **15**. The reaction of the thioester **15** (Segment A) with recombinant protein with N-terminal cysteine (Segment B) forms TMR-protein **16** (chromophore to protein ratio = 1) that can be purified by dialysis.

Please replace paragraph 0039 on pages 13-14 with the following:

FIG. 6 depicts a scheme showing site-specific modification of a protein that contains an N-terminal threonine or ~~cysteine~~ serine. The amino and hydroxyl groups on adjacent carbons of an N-terminal amino acid can be readily oxidized to form a protein with N-terminal aldehyde (**17**, Segment B). Coupling of Segment B to **19** (Segment A) results in a visibly colored protein (**21**) with known molecular weight and pI.

Please replace paragraph 0042 on page 14 with the following:

FIG. 9 depicts a scheme illustrating in vitro chemical ligation using a peptide without N-terminal cysteine. The ~~N^{α}-(1-phenyl-2-mercaptoethyl)~~ N_{α}-(1-(4-methoxybenzene)-2-mercaptoethyl) auxiliary is coupled to the oligopeptde N-terminus using solid phase peptide synthesis. Upon ligation, the auxiliary group is removed under mild conditions.

Please replace paragraph 0072 on pages 25-26 with the following:

Chemical ligation of proteins has the following advantages in the present invention:

- It is site-specific and allows only a single specific coupling reaction between the C_{α} of one segment (e.g., Segment A or Segment B) and N_{α} of another segment (e.g., Segment A or Segment B), in the presence of other reactive groups.
- It generates only one product.
- The resulting product has a known pI and a known molecular weight. These parameters can be determined theoretically and experimentally.
- It allows protein labeling using chromophores and fluorophores in a consistent, reproducible fashion.

- It allows nucleic acid labeling using chromophores and fluorophores in a consistent, reproducible fashion.
- It can be used to alter the pI of proteins. The incorporation of charged amino acid residues, or of charged chromophoric groups into Segment A, will alter the pI of the final protein product. For example, the guanidino group of arginine (pKa >12) will shift the pI of the product to basic pH, whereas, chromophores with a sulfonic acid group (pKa of 1.5) will shift the pI of the product to acidic pH. Other charged chromophores or charged amino acids will have similar effects.
- It allows manipulation of the molecular weight of proteins. For example, a 30-residue oligopeptide (Segment A) increases the molecular weight of the protein (Segment B) by approximately 3.0 <u>kilodaltons</u> ~~daltons~~ (kD), depending on the amino acid sequence, upon ligation.
- It allows incorporation of tags into proteins. Addition of tags such as biotin, fluorescein, digoxigenin, polyhistidine to the synthetic peptide followed by ligation of the peptide to the protein generates a tagged protein. This tagging strategy may be used to facilitate purification.
- It allows ligation of polynucleotides to labeled oligopeptides in a consistent, reproducible fashion.

Please replace paragraph 0085 on pages 32-33 with the following:

In another embodiment, Segment A may be a TMR-labeled organic thioester (see FIG. 3A). Acylation of triethylenetetramine (TREN, available from Aldrich, Milwaukee, WI, Catalogue No. 90462) with 3.5 equiv. of an activated ester of carboxytetramethylrhodamine (TMR), available from Molecular Probes, OR (Catalogue No. e-6123), forms $(TMR)_3$-TREN **5**. Acylation of N^{α}-*Fmoc*-Lysine with 2-iminobiotin-N-hydroxysuccinimide ester (Biotin-NS ester) yields N_{ε}-*Fmoc*-N_{α} -biotin-Lysine **6**, see FIGs. 3A and 3B. Deblocking of the ~~α-amino~~ <u>ε-amino</u> group of **6** followed by acylation with bromoacetyl chloride forms N_{ε}-bromoacetamido-N_{α}-biotinyl-Lysine **8**. The carbodiimide coupling of **8** with α-toluenethiol results in **9**. The alkylation of **5** with the thioester **9** in the presence of sodium iodide generates the quaternary ammonium salt **10**

(Segment A) that upon coupling with Segment B under the same conditions described above affords **11** (chromophore to protein ratio = 3).

Please replace paragraph 0114 on page 43 with the following:

Deblocking: A reaction mixture containing 1.364 g of Cys-Leu-Lys(TMR)-Asp-Ala-Leu-Asp-Ala-Leu-Asp-Ala-Leu-Lys(TMR)-Asp-Ala-resin (SEQ ID NO:3) was added with 300 μL of scavenger mixture (thioanisole 10 ml/~~triisopropylsiline~~ triisopropylsilane 4 ml/phenol 600 mg), 200 μl of mercaptopropionic acid (MPA) and 10 ml of 95% TFA/5% H_2O was left at room temperature for 3 hours with occasional stirring. A 100 ml of tert-butyl methyl ether (MTBE)/hexane (1:1) was added to the reaction mixture and centrifuged. The supernatant was decanted, and the residue was washed with 50 ml of MTBE/hexane (1:1) and centrifuged again. The solid was separated by decantation, extracted with 50 ml of 50% of acetonitrile in H_2O and lyophilized. The crude mixture was purified on preparative C-18 RP-HPLC to yield 198 mg of pure peptide that was MS analyzed by MS (Found 2397.67, Calc. 2398.71).